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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 09/601,644 | 12/11/2000 | Jean Gariepy | MMC.P-001 | 7797 |

21121 7590 07/19/2005

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| EXAMINER | |
|---|---|
| SHIBUYA, MARK LANCE | |
| **ART UNIT** | **PAPER NUMBER** |
| 1639 | |

DATE MAILED: 07/19/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 09/601,644 | GARIEPY ET AL. | |
| | Examiner | Art Unit | |
| | Mark L. Shibuya | 1639 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *03 March 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-18,20,24,25 and 27-42* is/are pending in the application.

4a) Of the above claim(s) *17,18,20,24,25 and 27-41* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-16 and 42* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *3/3/2005*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

## DETAILED ACTION

1. Claims 1-18, 20, 24, 25, 27-42 are pending. Claims 17, 18, 20, 24, 25, 27-41 are withdrawn from consideration. Claims 1-16 and 42 are examined.

### *Election/Restrictions*

2. Upon reconsideration, and in view of applicant's arguments entered 3/03/2005, claim 42 is rejoined to elected Group I, and is examined.

3. Claims 17-41 remain withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected species and inventions, there being no allowable generic or linking claim.

4. Applicant again traverses the restriction requirement in the response entered 3/3/2005. The traversal is on the ground(s) that there is no lack of unity for claims 1-42, because the references of Jackson et al., and Tyrrell et al., do not anticipate the invention as in claim 1. The examiner respectfully submits that applicant's arguments are not persuasive and that the references of Jackson et al., and Tyrrell et al., do anticipate the claimed invention. Please see the below rejections under 35 USC 102 (b).

### *Priority*

5. This application is a 371 of PCT/CA98/01137, filed 12/08/1998.

6. Acknowledgment is made of applicant's claim for foreign priority under 35 U.S.C. 119(a)-(d). The certified copy of Canadian priority document 2,222,993, filed on Feb. 4, 1998, appears in the instant application papers, and in PCT/CA98/01137, filed 12/08/1998.***Information Disclosure Statement***

7. The Information Disclosure Statement, entered 3/3/2005, has been considered.

***Nucleotide and Amino Acid Sequence Disclosures***

8. Applicant's submission of a new SEQ ID Listing identifying all nucleotide and amino acid sequences by Sequence Identifier; a new CRF copy of the new SEQ ID Listing; and a statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d), is acknowledged.

***Specification***

9. Applicant's amendment of the specification to identify any nucleotide and amino acid sequences by SEQ ID No. is acknowledged. The examiner notes with appreciation applicant's assistance in identifying and correcting all incidences of non-complying sequence disclosures in the specification.

10. The Drawings are objected to because Figure 1 appears to be missing from the drawings as filed 8/4/2000.

***Withdrawn Claim Rejections***

11. The rejection of claims 1-16 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention, is withdrawn in view of applicant's amendments to the claims, entered 3/3/2005.

***New Claim Rejections - 35 USC § 112***

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> **The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.**

12. Claims 1-16 and 42 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This rejection is for lack of written description.

The claims are drawn to a method for making a cytotoxic mutant protein having a different receptor-binding specificity than the wild type protein, comprising incorporating mutations into DNA encoding the binding domain of a heteromeric protein toxin to produce variant forms of the heteromeric protein toxin, generating a library of clones to produce variant forms of the heteromeric protein toxin, screening against a population of screening cells and selecting a cytotoxic mutant protein that inhibits or kills said population of screening cells to a greater extent than wild-type cytotoxic mutant protein.

Vas-Cath Inc. v. Mahurkar, 19 USPQ 2d 1111, clearly states "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession *of the invention*. The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed*." (See Vas-Cath at page 1117). The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed." (See Vas-Cath at page 116). The claimed

genus of cytotoxic proteins is broad and includes species named in the specification and claimed, such as Shiga toxin, Shiga-like toxins, ricin, abrin, gelonin, crotin, pokeweed antiviral protein, saporin, momordin, modeccin, sarcin, diphtheria toxin and Psudomonas aeruginosa exotonin A, as well as other species not described, such as snake, lizard, spider and insect venoms (see, e.g., US Patent No. 6,833,131 at col. 1, lines 10-35; US Publication No. 2002/0161203 A1 at para [0005], [0190]). The specification provides specific embodiments, working or otherwise, only for Shiga-toxin and Shiga-like toxins. In the Specification, at Example 4, pp. 22-23, the method used for producing a cytotoxic mutant protein having a different receptor-binding specificity than the wild type protein appears to rely on using the CAMA-I cell line, because it lacks the CD77 marker that is the receptor for Shiga toxin and Shiga-like toxin (p. 12, lines 16-19). The specification does not disclose cell lines that similarly lack the receptors for the other heteromeric protein toxins that constitute the genus. The examiner respectfully submits that the specification does not provide a representative number of species to show possession over the entire genus claimed. It is noted that the examined claims do not require that the screening cell line lack the receptor recognized by the wild-type toxin.

The specification at p. 25, lines 25-29, states that the "B subunit variants may thus bind to a spectrum of molecular entities such as proteins, peptides, nucleic acids or even organic moieties rather than to sugars or glycolipids (such as CD77)." However, in regard to the embodied species of Shiga-toxin, the specification does not describe what different receptor the mutated B subunit now has specificity for and describes no

assays, actual or prophetic, to demonstrate positively that the mutated toxins now have specificity for a different receptor, as claimed. Also, the specification does not disclose that the mutated B subunits do not bind to the CD77; rather that the mutated toxin kills CAMA-1 cells, which the specification teaches lacks CD77, and SKBR-3 cells, which the specification teaches expresses CD77 (Specification at p. 22, lines 4-14). Given the unpredictability of the arts of biology and of mutation, particularly in changing the target of a protein ligand, extrapolation from cytotoxicity data (see Specification at Example 4, pp. 22-23) that the mutated Shiga toxin B subunit has a different receptor-binding specificity is uncertain. The Office does not have the facilities and resources to provide the factual evidence needed in order to determine that the cytotoxic mutant protein has a different receptor-binding specificity than the wild type protein. It is respectfully submitted that the practitioner would not be reasonably apprised that the applicant was in possession of the claimed invention, in regard to the particular species of Shiga toxin or Shiga-like toxin.

Vas-Cath Inc. v. Mahurkar, 19 USPQ 2d 1111, clearly states "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession *of the invention*. The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed.*" (See Vas-Cath at page 1117). See, also, Fiers v. Revel, 25 USPQ 2d 1601 at 1606 (CAFC 1993) and Amgen Inc. v. Chugai Pharmaceutical Co. Ltd., 18 USPQ2d 1016. One cannot describe what one has not conceived. See Fiddes v. Baird, 30 USPQ2d 1481 at 1483. In Fiddes, claims directed to mammalian FGF's were found to be unpatentable due to lack of written

description for that broad class. The specification provided only the bovine sequence. Applicant is reminded that Vas-Cath makes clear that the written description provision of 35 § 112 is severable from its enablement provision.

13. Claims 1-16 and 42 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

There are many factors be considered when determining whether there is sufficient evidence to support a determination that a disclosure does not satisfy the enablement requirement and whether undue experiment is necessitated. These factors can include, but are not limited to:

(1) the breadth of the claims;
(2) the nature of the invention;
(3) the state of the prior art;
(4) the relative skill of those in the art;
(5) the level of predictability in the art;
(6) the amount of direction provided by the inventor;
(7) the existence of working examples; and
(8) the quantity of experimentation needed to make or use the invention based on the content of the disclosure.

In re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

(1 and 2) The breadth of the claims and the nature of the invention: The claims are drawn to a method for making a cytotoxic mutant protein having a different receptor-binding specificity than the wild type protein, comprising incorporating mutations into

DNA encoding the binding domain of a heteromeric protein toxin to produce variant forms of the heteromeric protein toxin, generating a library of clones to produce variant forms of the heteromeric protein toxin, screening against a population of screening cells and selecting a cytotoxic mutant protein that inhibits or kills said population of screening cells to a greater extent than wild-type cytotoxic mutant protein. Thus the claim is broadly drawn to any heteromeric protein toxin. The specification at p. 25, , lines 25-29, states that the "B subunit variants may thus bind to a spectrum of molecular entities such as proteins, peptides, nucleic acids or even organic moieties rather than to sugars or glycolipids (such as CD77)." Therefore, the different receptor to which the variant forms of the mutated cytotoxic protein can bind, is contemplated by the specification to encompass virtually any biological molecule. It is noted that the examined claims do not require that the screening cell line lack the receptor recognized by the wild-type toxin.

(3 and 5) The amount of direction provided by the inventor and the existence of working examples: Applicants have only exemplified the preparation of mutant Shiga toxin, although the example probably is applicable to Shiga-like Toxin-1, as the specification at p. 12, lines 16-19 teaches that both toxins recognize the glycolipid CD77 (also known as $Gb_3$). In Example 4, pp. 22-23, the specification provides specific embodiments, working or otherwise, only for method used for producing a cytotoxic mutant protein of Shiga-toxin. However, in regard to the embodied species of Shiga-toxin, the specification does not describe what different receptor the mutated B subunit now has specificity for and describes no assays, actual or prophetic, to demonstrate positively that the mutated toxins now have specificity for a different receptor, as

claimed. The specification does not disclose the molecule, if any, to which the mutated B subunit of the variant Shiga toxin protein now binds. Also, the specification does not disclose that the mutated B subunits do not bind to the CD77; rather that the mutated toxin kills CAMA-1 cells, which the specification teaches lacks CD77, and SKBR-3 cells, which the specification teaches expresses CD77 (Specification at p. 22, lines 4-14).

(4) The state of the prior art and the level of predictability in the art Methods for making for making mutant Shiga toxin and mutant Shiga-like toxin was known in the art at the time of filing; however, only a limited number of mutant Shiga toxin that have a specificity for a different receptor from that recognized by wild type Shiga toxin were known. Furthermore, the art of ligand mutation and receptor specificity is unpredictable. Applicant's claimed scope of any heteromeric protein toxin, such that mutations thereto that result in changed receptor specificity from that of wild type toxin, and such that a population of screening cells would be killed or inhibited to a greater degree, than by the wild type toxin, represent only an invitation to experiment (see also above concerning written description and cases cited therein). In view of the uncertainty in the art, extrapolation from cytotoxicity data (see Specification at Example 4, pp. 22-23) that the mutated Shiga toxin has a different receptor-binding specificity is unpredictable.

(6-7) The level of one or ordinary skill: The level of skill would be high, most likely at the Ph.D. level. However, such persons of ordinary skill in this art, *given its unpredictability*, would have to engage in undue (non-routine) experimentation to carry out the invention as claimed.

(8) The quantity of experimentation needed to make or use the invention based on the content of the disclosure: The claims contain only broad recitations of "heteromeric protein toxin" and mutant variant protein toxins having "a different receptor-binding specificity". However, the instant specification does not provide to one skilled in the art a reasonable amount of guidance with respect to the direction in which the experimentation should proceed in carrying out the full scope of the claimed methods. Note that there must be sufficient disclosure, either through illustrative examples or terminology, to teach those of ordinary skill how to make and use the invention as broadly as it is claimed. *In re Vaeck*, 947 F.2d 488, 496 and n.23, 20 USPQ2d 1438, 1455 and n.23 (Fed. Cir. 1991). Therefore, it is deemed that further research of an unpredictable nature would be necessary to make or use the invention as claimed. Thus, due to the inadequacies of the instant disclosure, undue experimentation would be required of one of ordinary skill in the art to practice the full scope of the claimed invention.

***New Claim Rejections - 35 USC § 112, Second Paragraph***

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

14. Claims 1-16 and 42 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1, and its dependent claims, recite the language "different receptor-binding specificity", which renders the claims vague and indefinite because the phrase might mean "specific for a different receptor", or "receptor binding that is different" (but where the receptor remains the same). Because the language is capable of more than one meaning, the claims are rendered indefinite.

***Maintained Claim Rejections - 35 USC § 102***

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

15. Claims 1-3, 5, 8-13, and 16 are rejected under 35 U.S.C. 102(b) as being anticipated by Jackson et al., J. of Bacteriology, Feb. 1990, Vol. 172, No. 2, pp. 653-658, (previously cited). This rejection maintains the reasons of record as set forth in the previous Office action.

The claims are drawn to a method for making a cytotoxic mutant protein or pool of proteins from a cytotoxic wild type protein said mutant protein or pool of proteins having a different receptor-binding specificity than the wild type protein, comprising: (A) selecting a heteromeric protein toxin having a toxic domain or subunit and a binding domain or subunit; (B) incorporating mutations into DNA encoding the binding domain or subunit of the heteromeric protein toxin to produce a plurality of variant forms of the heteromeric protein toxin; (C) generating a library of microorganism clones producing variant forms of the heteromeric protein toxin; D) screening the variant forms of the

heteromeric protein toxin of said library against a population of screening cells by isolating clones or pools of clones producing said variant forms of the heteromeric protein toxin, treating preparations of said population of screening cells with variant forms of the heteromeric protein toxin produced by the isolated clones or pools of clones, and selecting a cytotoxic mutant protein or pool of cytotoxic mutant proteins that inhibits or kills said population of screening cells to a greater extent than the wild-type cytotoxic mutant protein, whereby said selected mutant protein or pool of proteins has a different receptor binding specificity than the wild-type binding protein; and (E) making additional copies of the selected cytotoxic mutant protein or pool of proteins, and variations thereof.

Jackson et al., throughout the publication, and especially at the abstract, teach making a cytotoxic mutant protein from a cytotoxic wild type protein that is Shiga toxin and Shiga-like toxin, said mutant protein or pool of proteins, comprising: (A) selecting a Shiga toxin or Shiga-like toxin having an enzymatic subunit A, reading on a toxic domain or subunit and a binding subunit B; (B) incorporating mutations into DNA encoding the binding subunit B of Shiga toxin and Shiga-like toxin protein toxin to produce a plurality of variant forms of the heteromeric protein toxin, (Specification at p. 654, para 1 and Table 1, p. 655); (C) generating a library of microorganism clones producing variant forms of the heteromeric protein toxin, (Specification at p. 654, para 2 and Table 1, p. 655); D) screening the variant forms of the heteromeric protein toxin of said library against a population of screening cells that are Vero cells or HeLa cells, as in Table 2, p.656 (see also p. 654-655, bridging paragraph, "Microcytotoxicity Assay"),

by isolating clones or pools of clones producing said variant forms of the heteromeric protein toxin (p. 654-655, bridging paragraph, "Microcytotoxicity Assay"), treating preparations of said population of screening cells with variant forms of the heteromeric protein toxin, (e.g., D16N, D17N, as in Table 2, and compared to wild type protein toxin pEW 3.0); and (E) making additional copies of the selected cytotoxic mutant protein or pool of proteins (Table 4, p. 657, para 1).

Jackson et al. teach mutations to the B subunit of Shiga toxin and Shiga-like toxin. The Office does not have the facilities and resources to provide the factual evidence needed in order to determine whether the mutant protein toxin variant disclosed by Jackson et al. have a different receptor-binding specificity than the wild type protein.

*Response to Arguments*

Applicant argues that Jackson does not teach proteins that kill or inhibit cells to a greater extent than the wild-type and thus there can be no selection of such proteins or copying of such proteins.

Applicant's arguments filed 3/3/2005 have been fully considered but they are not persuasive. It is to be expected that in a screening step, there will be mutant proteins that do not cause inhibition of killing of screening cells to a greater extent than by wild type protein toxin. Because Jackson et al. teach the method steps of the claimed invention, the procedure of Jackson anticipates the claimed process. It is up to applicant to prove that the process of Jackson would not perform the claimed method. See, MPEP 2112.02. Applicant has failed to introduce any evidence that the method of

Jackson et al. would not result in proteins that are show to kill or inhibit cells to a greater extent than the wild-type.

16. Claims 1-3, 5, 8-13, and 16 are rejected under 35 U.S.C. 102(b) as being anticipated by Tyrrell et al., Proc. Natl. Acad. Sci. USA, vol. 89, pp. 524-528, Jan 1992 (IDS filed 11/20/2000). This rejection maintains the reasons of record as set forth in the previous Office action.

Tyrrell et al., throughout the publication, and especially at the abstract, p. 524, teach the verotoxin, or Shiga-like toxin, family is a group of subunit toxins, with an A subunit and a B binding subunit and teach the site-directed mutagenesis of the B subunit; teach generating a library of E. coli that produced variant forms of the toxin, wherein the variant toxins include a mutation at position 18, among other targeted amino acids, as shown in fig. 1 and Table 1; wherein microorganisms were expanded and the variant toxins that each clone produces were extracted and screened in cytotoxicity assays using Vero cells, MRC-5 cells and HEp-2 tumor cells; and at p. 528, Fig. 4, teach the mutant toxin GT3 kills more HEp-2 cells than the wild-type toxin. Tyrrell, at, for example, the abstract, teach mutating the B subunit of Shiga-like family toxin that results in a change in the glycosphingolipid receptor specificity.

*Response to Arguments*

Applicant argues that the mutant of Tyrell et al. were made by specific introduction of mutations, not by creating a library, and that there is no screening for higher activity from a library of mutants.

Applicant's arguments filed 3/3/2005 have been fully considered but they are not persuasive. Applicant claims a "library" of microorganisms comprising mutant toxic proteins; however, the term "library" is used quite broadly in the art to mean "any ensemble of molecules" (e.g., see Janda, K.D. "Tagged versus untagged libraries: Methods for the generation and screening of combinatorial chemical libraries", PNAS USA November 1994, 91, 10779-10785, especially page 10779, column 1, last sentence, "in its purest form, a combinatorial chemical library can be defined as any ensemble of molecules"). As there is no specific definition of a library in applicant's specification, any ensemble of microorganisms comprising molecules that read on those set forth in the claims is deemed to be a library. The reference of Tyrrell et al. teaches a library, as evidenced by Janda. The mutant proteins of Tyrrell et al. clearly read on those claimed as set forth, the reference is deemed to disclose a "library".

***Maintained Claim Rejections - 35 USC § 103***

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

17. Claims 1 and 4 are rejected under 35 U.S.C. 103(a) as being unpatentable over either of Jackson et al., J. of Bacteriology, Feb. 1990, Vol. 172, No. 2, pp. 653-658, (previously cited with the Requirement for Restriction/Election, mailed 8/10/04) or Tyrrell et al., Proc. Natl. Acad. Sci. USA, vol. 89, pp. 524-528, Jan 1992 (IDS filed 11/20/2000),

each taken separately, and Cheng et al., (US 5,869,250). This rejection maintains the reasons of record as set forth in the previous Office action.

*Response to Arguments*

Applicant argues that the examiner has not said where in the teaching of the primary references this modification would be made, or what features of the references, as opposed to the terms of the present claims, would have suggested such a modification. Applicant states that no factual inquiry is demonstrated by the examiner, and thus no valid *prima facie* rejection.

Applicant's arguments filed 3/3/2005 have been fully considered but they are not persuasive. The reference of Jackson et al. seeks to identify the domains responsible for receptor binding by using libraries of mutation within those domains, (see abstract), so that one of ordinary skill in the art would have been motivated to use yeast libraries because Cheng et al. at col. 4, lines 31-44, teach that yeast can express a large number of peptides from combinatorial libraries from which desired peptides can be selected.

The reference of Tyrrell et al. investigates the role of amino acid substitutions in determining carbohydrate receptor binding specificity (at p. 524, para 1) so that one of ordinary skill in the art would have been motivated to use yeast libraries because Cheng et al. at col. 4, lines 31-44, teach that yeast can express a large number of peptides from combinatorial libraries from which desired peptides can be selected.

18. Claims 1 and 6 are rejected under 35 U.S.C. 103(a) as being unpatentable over either of Jackson et al., J. of Bacteriology, Feb. 1990, Vol. 172, No. 2, pp. 653-658,

(previously cited with the Requirement for Restriction/Election, mailed 8/10/04) or Tyrrell et al., Proc. Natl. Acad. Sci. USA, vol. 89, pp. 524-528, Jan 1992 (IDS filed 11/20/2000), each taken separately, and Reidhaar-Olson et al., Meth Enzymol (1991), vol. 208: 564-586. This rejection maintains the reasons of record as set forth in the previous Office action.

*Response to Arguments*

Applicant argues that, again, the rejection is nothing more than an assertion, without reasoning or support, that using the cassette mutagenesis method of Reidhaar-Olson in combination with the primary references would have been obvious. Applicant argues that such an assertion is not sufficient, particularly since neither Jackson nor Tyrrell use random mutagenesis.

Applicant's arguments filed 3/3/2005 have been fully considered but they are not persuasive. The reference of Jackson et al. seeks to identify the domains responsible for receptor binding by using libraries of site-directed mutation within those domains, (see abstract), so that one of ordinary skill in the art would have been motivated to use combinatorial cassettes because Reidhaar-Olson et al. at p. 564, first paragraph, teach that "[i]nvestigations of protein structure and function often rely on the analysis of mutant proteins. With the advent of methods for rapid and economical chemical synthesis of DNA, there has been a steadily increasing use of *oligonucleotide-directed mutagenesis and cassette mutagenesis* to create specific mutations at particular sites, (emphasis added)."

The reference of Tyrrell et al. investigates the role of site-specific amino acid substitutions in determining carbohydrate receptor binding specificity (at p. 524, para 1) so that one of ordinary skill in the art would have been motivated to use combinatorial cassettes because Reidhaar-Olson et al. at p. 564, first paragraph, teach that "[i]nvestigations of protein structure and function often rely on the analysis of mutant proteins. With the advent of methods for rapid and economical chemical synthesis of DNA, there has been a steadily increasing use of *oligonucleotide-directed mutagenesis and cassette mutagenesis* to create specific mutations at particular sites, (emphasis added)."

19. Claims 1 and 7 rejected under 35 U.S.C. 103(a) as being unpatentable over either of Jackson et al., J. of Bacteriology, Feb. 1990, Vol. 172, No. 2, pp. 653-658, (previously cited with the Requirement for Restriction/Election, mailed 8/10/04) or Tyrrell et al., Proc. Natl. Acad. Sci. USA, vol. 89, pp. 524-528, Jan 1992 (IDS filed 11/20/2000), each taken separately, and Nickoloff et al., (US 5,354,670). This rejection maintains the reasons of record as set forth in the previous Office action.

*Response to Arguments*

Applicant argues that Nickoloff is cited merely for disclosure of a mutagenesis technique. Thus, Nickoloff does not overcome the fundamental deficiencies of the Jackson and Tyrrell references.

Applicant's arguments filed 3/3/2005 have been fully considered but they are not persuasive. The examiner respectfully submits that the references of Jackson and

Tyrrell are *not* deficient. Please see above rejections maintained under 35 USC 102(b). Nickoloff has been cited for the reasons of record as set forth in the previous Office action.

20. Claims 1, 2, and 13-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over either of **Jackson et al.**, J. of Bacteriology, Feb. 1990, Vol. 172, No. 2, pp. 653-658, (previously cited with the Requirement for Restriction/Election, mailed 8/10/04) or **Tyrrell et al.**, Proc. Natl. Acad. Sci. USA, vol. 89, pp. 524-528, Jan 1992 (IDS filed 11/20/2000), each taken separately, and **Frankel et al.**, (US 4,753,894). This rejection maintains the reasons of record as set forth in the previous Office action.

Claims 1, 2, and 13-15 are drawn to the method of claim 1 wherein the screening cells are breast cancer cells and that are SK BR-3 or CAMA-I. Claim 42 is drawn to the method of claim 1, wherein the screening cells are insensitive to the wild-type cytotoxic protein.

**Jackson et al.**, throughout the publication, and especially at the abstract, teach making a cytotoxic mutant protein from a cytotoxic wild type protein that is Shiga toxin and Shiga-like toxin, said mutant protein or pool of proteins, comprising: (A) selecting a Shiga toxin or Shiga-like toxin having an enzymatic subunit A, reading on a toxic domain or subunit and a binding subunit B; (B) incorporating mutations into DNA encoding the binding subunit B of Shiga toxin and Shiga-like toxin protein toxin to produce a plurality of variant forms of the heteromeric protein toxin, (Specification at p. 654, para 1 and Table 1, p. 655); (C) generating a library of microorganism clones

producing variant forms of the heteromeric protein toxin, (Specification at p. 654, para 2 and Table 1, p. 655); D) screening the variant forms of the heteromeric protein toxin of said library against a population of screening cells that are Vero cells or HeLa cells, as in Table 2, p.656 (see also p. 654-655, bridging paragraph, "Microcytotoxicity Assay"), by isolating clones or pools of clones producing said variant forms of the heteromeric protein toxin (p. 654-655, bridging paragraph, "Microcytotoxicity Assay"), treating preparations of said population of screening cells with variant forms of the heteromeric protein toxin, (e.g., D16N, D17N, as in Table 2, and compared to wild type protein toxin pEW 3.0); and (E) making additional copies of the selected cytotoxic mutant protein or pool of proteins (Table 4, p. 657, para 1).

**Tyrrell et al.**, throughout the publication, and especially at the abstract, p. 524, teach the verotoxin, or Shiga-like toxin, family is a group of subunit toxins, with an A subunit and a B binding subunit and teach the site-directed mutagenesis of the B subunit; teach generating a library of E. coli that produced variant forms of the toxin, wherein the variant toxins include a mutation at position 18, among other targeted amino acids, as shown in fig. 1 and Table 1; wherein microorganisms were expanded and the variant toxins that each clone produces were extracted and screened in cytotoxicity assays using Vero cells, MRC-5 cells and HEp-2 tumor cells; and at p. 528, Fig. 4, teach the mutant toxin GT3 kills more HEp-2 cells than the wild-type toxin. Tyrrell, at, for example, the abstract, teach mutating the B subunit of Shiga-like family toxin that results in a change in the glycosphingolipid receptor specificity.

Jackson and Tyrrell et al., each taken separately, do not teach screening cells that are breast cancer cells and are SK BR-3 or CAMA-I.

**Frankel et al.**, (US 4,753,894) et al., throughout the patent, and especially at col. 3, lines 46-57, teach testing antibodies (where different antibodies have different antigen specificities) conjugated to ricin toxin in cytotoxicity assays using CAMA-1 and SKBR-3 breast tumor cells.

It would have been *prima facie* obvious at the time the invention was made for one of ordinary skill in the art to have tested cytotoxic mutant proteins in cytotoxicity assays comprising CAMA-1 and SKBR-3 breast tumor cells, and wherein the screening cells are insensitive to the wild-type cytotoxic protein, (as in claim 42), because CAMA-1 inherently lacks the CD77 marker, as evidenced by the Specification at p. 12, lines 16-19.

One of ordinary skill in the art would have been motivated to screen cytotoxic mutant proteins having different receptor-binding specificity than the wild type protein because CAMA-1 and SKBR-3 breast tumor cells are an art-recognized *in vitro* model system for selecting anti-cancer agents, including those comprising a ricin protein toxin, as taught by Frankel et al.

*Response to Arguments*

Applicant argues that the reference of Frankel teaches breast cancer-specific monoclonal antibodies conjugated to ricin as a cytotoxic agent for use in killing breast cancer cells, so that the reference of Frankel has nothing to do with the development of

toxins, but rather with the development of antibodies. Therefore, Frankel is non-analogous art, and should not be relied upon.

Applicant's arguments filed 3/3/2005 have been fully considered but they are not persuasive. In response to applicant's argument that the reference of Frankel is nonanalogous art, it has been held that a prior art reference must either be in the field of applicant's endeavor or, if not, then be reasonably pertinent to the particular problem with which the applicant was concerned, in order to be relied upon as a basis for rejection of the claimed invention. See *In re Oetiker*, 977 F.2d 1443, 24 USPQ2d 1443 (Fed. Cir. 1992). In this case, applicant's field of endeavor, and the particular problem with which applicant was concerned, was changing the receptor specificity of a protein toxin by mutation, such that screening cells would be killed or inhibited to an extent greater than that shown by the wild type toxin. The patent of Frankel contemplates antibodies with different receptor specificity, such that breast cancer cells would be killed to a greater extent (depending upon receptor specificity), when the antibodies were conjugated to a protein toxin that is ricin. It is noted that ricin is recited in instant claim 9. Thus Frankel is analogous art, in that Frankel, as in the claimed invention, is concerned with protein toxin subunits targeted by protein ligand subunits with different receptor specificities, and wherein the receptors are found on screening cells that are CAMA-1 and SK BR-3 breast cancer cells, as in instant claim 15.

***Conclusion***

21. Claims 1-16 and 42 are rejected.

22. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Mark L. Shibuya whose telephone number is (571) 272-0806. The examiner can normally be reached on M-F, 8:30AM-5:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Andrew Wang can be reached on (571) 272-0811. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Mark L. Shibuya
Examiner
Art Unit 1639

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